domain or fragment of said antibody demonstrating increased hydrophilicity as compared to said domain or fragment of said parent antibody in unmodified form.

38. A DNA sequence according to claim 37, comprising (i) a modification of an inter-domain interface in said variable heavy domain, as compared to a domain or fragment of a parent antibody; and (ii) a modification of an inter-domain interface in said variable light domain, as compared to a domain or fragment of a parent antibody.

REMARKS

Introduction

Claims 1-5 and 7-27 are pending in the present application. Upon entry of the foregoing amendment, claims 1, 2 and 12 will be amended and claims 37 and 38 will be added. None of the amendments narrow the scope of the invention. Applicants note with appreciation the Examiner's indication that claim 12 contains allowable subject matter.

In the May 31, 2002 Office Action, the drawings and the previously submitted substitute specification were objected to. Claims 1-5, 7-11, 13-17, and 26-27 were rejected under 35 USC §102(b) as anticipated by Johnson *et al.* ("Johnson"). Claims 1-5, 7, 10-11, and 13-27 were rejected under 35 USC §103(a) as obvious over Johnson in view of Jenkins *et al.* ("Jenkins"), Knappik *et al.* ("Knappik"), Dubel *et al.* ("Dubel") and Kostelny *et al.* ("Kostelny"). The specific grounds for rejection, and applicants response thereto, are described in detail below. Claims 1-5, 7-27 and 37-38, including independent claims 1 and 37, are thus pending for reexamination and reconsideration, which are respectfully requested in view of the foregoing amendments and following remarks.

Support for Amendments

Support for the "inter-domain" recitation of claims 1 and 12 can be found, *e.g.*, in the attached substitute specification (clean copy) at page 4, line 5. Support for the "functional" recitation in claim 1 can be found, inter alia, in the attached substitute specification (clean copy) at page 19, line 32 through page 20, line 3. The amendment to claim 2 simply moves the phrase "with amino acids which are more hydrophilic" to a different location for simplicity in reading. Support for new claims 37 and 38 can be found in attached substitute specification (clean copy) at page 4, line 5, as well as page 8, lines 15-16.

Objections to the Specification

The substitute specification filing of January 22, 2002, allegedly did not contain a marked-up copy of the specification. Accordingly, the amended substitute specification was not entered. Applicants submit herewith (i) a clean copy of an amended specification and (ii) a marked-up copy indicating all changes.

The substitute specification contains all amendments made through the October 22, 2001 Reply, plus additional changes. The additional changes relate to: (i) correcting additional typographical errors and (ii) converting certain portions of Figures 2(a) (pages 5-10 of the figures) and 2(b) (pages 14-19 of the figures) to tables 4 and 5, respectively. Applicants hereby state that the substitute specification contains no new matter, as required by 37 C.F.R. § 1.125 (b)(1). Accordingly, entry of the substitute specification and withdrawal of the objection are respectfully requested.

Objections to the Figures

The draftsperson in charge of this application has objected to figure 1 and certain portions of figures 2a and 2b. Applicants submit herewith a corrected figure 1, which is believed to overcome the objection. Applicants have obviated the objections to figures 2a and 2b by moving the objected-to portions to the specification as tables 4 and 5, respectively. Therefore, withdrawal of all objections is solicited.

Rejections under 35 U.S.C. § 102(b)

Claims 1-5, 7-11, 13-17, and 26-27 are rejected under 35 U.S.C. 102(b) as anticipated by Johnson. Applicants respectfully traverse on the grounds that Johnson does not teach a DNA sequence encoding a domain of an antibody that contains a modification of an inter-domain interface of the antibody, as required by all pending claims. Accordingly, Johnson does not recite each and every element of the claimed invention, and withdrawal of the rejection respectfully is withdrawn.

To more clearly demonstrate the distinction between the instantly claimed invention and the molecules described by Johnson, applicants respectfully submit that a brief description of Johnson is required. Briefly, Johnson describes possible solutions to well-known problems with "single domain antibodies." These molecules contain a single antibody variable domain (usually a single VH domain) and lack the complementary antibody variable domain (for example a VL domain that is complementary to the VH domain). In some circumstances, single domain antibodies can retain some of the binding characteristics of the intact VH-VL pair. See Johnson at pages 1-2. However, in practice, single domain antibodies suffer from poor or inefficient

folding and high levels of non-specific binding that "disappointingly limit their utility." Johnson at page 2, line 5.

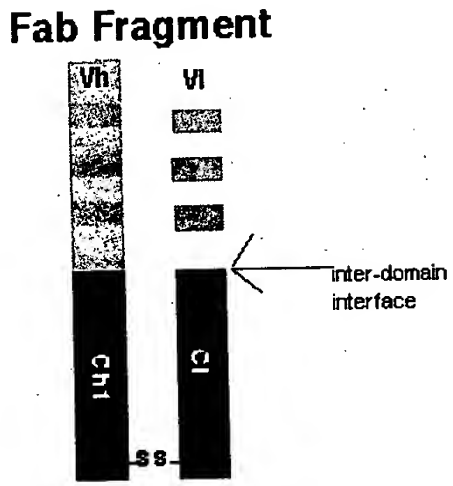
In antibodies or antibody fragments, the VH/VL pairing is facilitated by binding of a hydrophobic region on the VH chain with a complementary hydrophobic region on the VL chain. In a single variable domain molecule, this hydrophobic region is exposed to aqueous solvent, which is highly energetically unfavorable, leading to poor folding and promoting non-specific binding. See Johnson at page 3. Johnson attempts to solve these problems by identifying the amino acid residues that form the hydrophobic region in the single domain antibody and mutating those residues to less hydrophobic amino acids. See Johnson at pages 6-7. This replacement of amino acid residues in the hydrophobic region of the single variable domain is described in the instant specification which states that Johnson describes modifying amino acid residues in

> the region on a given heavy or light chain of an immunoglobulin which associates with the complementary heavy or light chain.

(see specification at page 13, lines 19-22) (emphasis added). In other words, Johnson describes modification of amino acid residues that would form the hydrophobic interface between the single chain variable domain and a complementary heavy or light chain variable domain.

By contrast, the instantly claimed invention is directed to DNA sequences encoding domains having modified residues on the "inter-domain" interface, rather than on the interface between a heavy and light chain variable region pair. As described in the instant specification (see, e.g., page 4, lines 1-7), it is apparent that an "inter-domain interface" exists within a single V_L/C_L chain or a single V_H/C_{H1} chain (for example between the "bottom" of V_L and the "top" of

C_L). The following drawing shows one representative example of an inter-domain interface (here, between a V_L and C_L in a Fab fragment).



Accordingly, it is clear that, in the context of the instantly claimed invention, an "inter-domain" interface is distinct from a "complementary" interface. Johnson does not describe modification of an inter-domain interface and, accordingly, does not describe each and every element of the instantly claimed invention and withdrawal of the rejection respectfully is requested.

Furthermore, new claims 37 and 38 require the presence of both a variable heavy chain and a variable light chain domain. Johnson, by contrast, teaches the presence of only one variable domain and, hence, cannot anticipate these claims. An indication of allowability of these claims over Johnson respectfully is requested.

There appears to be confusion on the record of the instant application regarding whether Johnson describes the use of "modified" scFv's. Although applicants reiterate that certain of the pending claims encompass DNA molecules encoding scFv's having one or more modified inter-

domain interfaces, it is readily apparent that Johnson fails to describe such modified scFv molecules.

Thus, as described above, a straightforward reading of Johnson reveals that the "modifications" undertaken therein are aimed at solving problems that occur when, for example, a VH domain is produced in the absence of a complementary VL domain:

> the present invention seeks to ameliorate... problems associated with single variable domain binding members whilst retaining antigen binding

See Johnson at page 2, line 27 through page 3, line 2) (emphasis added). A single variable domain in the absence of a complementary variable domain cannot produce a functional scFv which, by definition, requires the presence of both a VL and a VH domain which can associate with each other. Although Johnson refers to scFv molecules at page 13, lines 2-3 , nowhere does Johnson describe preparation of functional scFv molecules having modified amino acid residues.

Moreover, one skilled in the art would recognize that the modifications described by Johnson would not allow production of functional antibody fragments containing complementary VH and VL domains. Thus, the modifications described by Johnson would not only reduce non-specific hydrophobic interactions (as Johnson intended) but would also remove the specific hydrophobic interactions between complementary VH and VL domains, thereby promoting dissociation between the VH and VL domains and destabilizing the antibody fragment. Indeed, Johnson specifically describes altering V_H amino acid residues 37, 39, 45, 47, 91, 93 and 103, since these amino acid residues ordinarily interact with the complementary V_L domain (page 23, lines 4-6). A skilled worker in the field would conclude that the V_L and V_H domains of a so-called "scFv" modified in this way would fail to associate. Accordingly, an "scFv" constructed in accordance with the teachings of Johnson would not function as an scFv.

In sum, for the reasons described above, Johnson does not describe the modification of amino acids at an inter-domain interface of an antibody domain. Accordingly, Johnson fails to describe each and every element of the claimed invention, and withdrawal of the rejection is respectfully solicited.

Rejections under 35 U.S.C. § 103(a):

Claims 1-5, 7, 10-11, 13-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Johnson et al (WO 92/01787), in view of Jenkins et al. (PNAS 92:6057-6061, 1995) and Knappik et al. (Biotechniques 17(4):754-761, 1994), further in view of Dubel et al. (J. Immunol. Meth. 178:201-209, 1995) and Kostelny et al. (J. Immunol. 148:1547-1533, 1992). Applicants respectfully traverse the rejection on the grounds that a prima facie case of obviousness has not be made.

For the reasons described in detail above, Johnson fails to teach or suggest DNA molecules encoding a domain of a functional antibody or fragment thereof where the domain contains modifications of the amino acid(s) at an inter-domain interface. Rather, Johnson suggests, at most, the modification of amino acids at the large complementary interface of a single antibody variable domain. Nothing in Johnson suggests that a small inter-domain interface even exists, let alone that the modification of this small interface would unexpectedly increase solubility and yield as described at page 4 of the instant specification. None of the secondary references make up for the deficiencies of Johnson, or would have suggested to one of ordinary skill in the art that modifying the teachings of Johnson would somehow lead to the claimed invention. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 103(a).

CONCLUSION:

In view of the foregoing, applicants respectfully request the Examiner to withdraw each rejection and pass the claims to allowance. The Examiner is invited to contact the undersigned attorney to resolve any issues, in order to expedite the prosecution of the application.

Respectfully submitted,

August 29, 2002
Date

Paul M. Booth
Reg. No. 40,244

Customer ID No. 26633
HELLER EHRMAN WHITE & McAULIFFE
1666 K Street, NW, Suite 300
Washington, DC 20006-1228
(202) 912-2000 (telephone)
(202) 912-2020 (telecopier)

Marked Up Copy of Claims

1. (Five times amended) A DNA sequence [capable of] encoding a domain [or fragment of an] of a functional antibody or functional fragment thereof, [wherein said domain or fragment comprises an exposed] comprising a modification of an inter-domain interface [wherein:

a) said exposed interface allows contact along a longitudinal axis between adjacent domains within a heavy chain or within a light chain of a larger antibody fragment or full antibody;

b) said exposed interface comprises a modification] as compared to a domain or fragment of a parent antibody wherein said modification [to said exposed interface] results in said domain or fragment of said antibody demonstrating increased hydrophilicity as compared to said domain or fragment of said parent antibody in unmodified form..

2. (Amended) The DNA sequence according to claim 1 in which said modification is a substitution of one or more amino acids with amino acids which are more hydrophilic at said region which comprised or would comprise the inter-domain interface [with amino acids which are more hydrophilic].

3. The DNA sequence according to claim 1 in which said modification comprises:

a) insertion of one or more hydrophilic amino acids

b) insertion of one or more amino acids;

c) deletion of one or more hydrophobic amino acids; or

d) deletion of amino acids.

4. (Three times amended) The DNA sequence according to claim 1 in which said modification consists of any two or more of:

a) substitution of one or more amino acids at said region which comprised or would comprise the interface with amino acids which are more hydrophilic than the one or more amino acids being substituted for;

b) insertion of one or more hydrophilic amino acids or insertion of amino acids; and

c) deletion of one or more hydrophobic amino acids or deletion of amino acids.

5. The DNA sequence according to any of claims 2 to 4 in which said substituted or inserted amino acid is selected from the group consisting of Asn, Asp, Arg, Gln, Glu, Gly, His, Lys, Ser, and Thr.

6. (Canceled)

7. (Amended) The DNA sequence according to claim 1 in which said domain or fragment is derived from an antibody.

8. (Amended) The DNA sequence according to claim 1 in which said fragment is a Fab fragment.

9. (Amended) The DNA sequence according to claim 1 in which said fragment is an Fv fragment.

10. (Amended) The DNA sequence according to claim 1 in which said fragment is a scFv fragment.

11. (Amended) The DNA sequence according to claim 1 in which said fragment is an Fv stabilized by an inter-domain disulphide bond.

12. (Twice Amended) The DNA sequence according to any of claims 9 to 11 in which said [exposed] inter-domain interface comprises residues 9, 10, 12, 15, 39, 40, 41, 80, 81, 83, 103, 105, 106, 106A, 107, 108 for VL, and residues 9, 10, 11, 13, 14, 41, 42, 43, 84, 87, 89, 105, 108, 110, 112, 113 for VH.

13. The DNA sequence according to claim 1, having a contiguous sequence which encodes one or more additional moieties.

14. The DNA sequence according to claim 13 in which at least one of said additional moieties is a toxin, a cytokine, or a reporter enzyme.

15. The DNA sequence according to claim 13 in which at least one of said additional moieties is at least part of a surface protein of an organism.

16. The DNA sequence according to claim 15 in which said organism is a filamentous bacteriophage.

17. The DNA sequence according to claim 16 in which said surface protein is the gene III protein.

18. The DNA sequence according to claim 13 in which at least one of said additional moieties is capable of binding a metal ion.

19. The DNA sequence according to claim 18 in which at least one of said additional moieties comprises at least five histidines.

20. The DNA sequence according to claim 13 in which said moiety is a peptide.

21. The DNA sequence according to claim 20 in which said peptide is a labeling tag.

22. The DNA sequence according to claim 21 in which said labeling tag is c-myc or FLAG.

23. The DNA sequence according to claim 20 in which said peptide comprises an association domain which results in self-association of two or more of said antibody fragments.

24. The DNA sequence according to claim 23 in which said association domain is derived from a leucine zipper or from a helix-turn-helix motif.

25. The DNA sequence according to claim 20 in which said peptide comprises a first association domain which results in hetero-association of one or more of said antibody fragments with one or more peptides or proteins comprising a second hetero-association domain being able to associate with said first hetero-association domain.

26. A vector comprising a DNA sequence according to claim 1.

27. A host cell comprising a vector according to claim 26.

Claims 28-36 (Canceled) With Amendment of 12/1/99

37. (new) A DNA sequence encoding a domain of a functional antibody or a functional fragment thereof, comprising (i) a variable heavy domain, (ii) a variable light domain, and (iii) a modification of an inter-domain interface in said variable heavy or said variable light domain, as compared to a domain or fragment of a parent antibody, wherein said modification results in said domain or fragment of said antibody demonstrating increased hydrophilicity as compared to said domain or fragment of said parent antibody in unmodified form.

38. (new) A DNA sequence according to claim 37, comprising (i) a modification of an inter-domain interface in said variable heavy domain, as compared to a domain or fragment of a parent antibody; and (ii) a modification of an inter-domain interface in said variable light domain, as compared to a domain or fragment of a parent antibody.